BIOSYNTHESIS AND OPTIMIZATION OF METHYL 3-(3,5-DI-TERT-BUTYL-4-HYDROXYPHENYL) PROPIONATE PRODUCTION FROM OIL PALM FROND JUICE BY *Ceratocystis fimbriata*

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NANG NOR AZIMAH LONG NADZRI

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This page is entirely dedicated to……

……… my lovely mother and father (Zakiah Bt Awang and Long Nadzri Bin Long Hussin), husband (Mohammad Firdaus Bin Abu Bakar), family and friends who have always been at my side and given me the encouragement and support that carries me through my study. Thanks for their never-ending love, support and care to me……

(May ALLAH S.W.T. always be with all of you)

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Peace and Blessings be Upon His Beloved Prophet.

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ABSTRAK

Di Malaysia, ladang kelapa sawit dan industri minyak sawit merupakan penyumbang utama kepada pembentukan sisa pertanian. Dari kajian terdahulu, penyelidikan telah dijalankan untuk mengkaji potensi penggunaan sisa pertanian dengan cekap. Dalam kajian ini, jus kelapa sawit (OPF) digunakan untuk menggantikan fungsi glukosa semasa proses penapaian. Jus OPF dilaporkan mengandungi gula boleh diperbaharui seperti glukosa, sukrosa dan fruktosa. Jus OPF dijangka boleh menangani isu-isu alam sekitar untuk menghasilkan sebatian organik mudah meruap (VOC) terutamanya metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat kerana OPF banyak terbuang sebagai biojisim dan mudah diperoleh di seluruh Malaysia. Kajian penggunaan dan pembangunan penghasilan metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat dari kulat semakin meningkat kerana ia boleh dihasilkan secara semula jadi tanpa sintesis kimia. *Ceratocystis fimbriata* adalah kulat yang mempunyai potensi untuk mensintesis ester, ia tumbuh dengan cepat dan menghasilkan pelbagai aroma (pic, nanas, pisang, sitrus dan ros) bergantung kepada keadaan persekitaran dan kultur yang digunakan. Tujuan utama kajian ini adalah untuk menyaring dan mengoptimumkan penghasilan metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat dengan menggunakan satu siri reka bentuk eksperimen dengan teknik pengekstrakan fasa pepejal ruang (HS-SPME) dengan menggunakan gas kromotografi-spektroskopi jisim (GC-MS) untuk memisahkan kawasan puncak relatif sebatian selepas proses penapaian. Pengoptimuman penghasilan metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat dipengaruhi oleh beberapa faktor semasa proses penapaian. Siri reka bentuk eksperimen digunakan untuk menyaring dan mengoptimumkan pengeluaran sebatian itu. Dalam kajian penyaringan, kaedah rekabentuk faketorial penuh 2^4 telah digunakan untuk mencari faktor-faktor penting yang mempengaruhi pengeluaran metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat, yang merupakan suhu inkubasi (25 °C - 35 °C), medium pH awal (pH4 - pH 8), kelajuan agitasi (100 rpm - 150 rpm) dan kepekatan glukosa (20 g/L - 30 g/L) dalam jus OPF. Respon dalam penapisan dipadankan dengan persamaan regresi linear berganda dan memperoleh korelasi (R^2 = 0.8960) antara data eksperimen dan data model. Reka bentuk komposit pusat (CCD) digunakan sebagai reka bentuk eksperimen dan model regresi polinomial dengan istilah kuadrat digunakan untuk menganalisis data eksperimen menggunakan analisis varians (ANOVA). Analisis ANOVA menunjukkan bahawa model sangat signifikan ($p < 0.0001$) untuk menghasilkan metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat. Respon tersebut dipadankan dengan persamaan polinomial urutan kedua dengan korelasi tinggi (R^2 = 0.9598) di antara nilai ujikaji dan nilai yang diramalkan. Keputusan proses pengoptimuman menunjukkan bahawa penghasilan propionat maksimum metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat diperolehi dalam keadaan medium pH awal (8), kelajuan agitasi (100 rpm) dan inkubasi suhu (25 ° C). Di bawah keadaan optimum ini, pengeluaran metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat tertinggi didapati apabila masa penahanan adalah pada 32.80 minit dan kawasan puncak relatif 0.29% kawasan kromatogram dengan menggunakan GC-SPME. Kesimpulan kajian ini telah memberikan garis panduan yang signifikan dan kefahaman awal bagi penghasilan sebatian metil 3- (3,5-di-tert-butil-4-hidroksifenil) propionat dengan menggunakan jus OPF sebagai substrat tunggal, boleh diperolehi dan mampan oleh *C. fimbriata* pada skala yang lebih besar pada masa hadapan.

ABSTRACT

In Malaysia, oil palm plantations and the palm oil industries were the main contributors to the generation of agricultural waste. From previous studies, researchers have identified the potential of utilizing the agricultural waste efficiently. In this research, oil palm frond (OPF) juice was used to replace the function of glucose during fermentation. OPF juice is reported to contain renewable sugars such as glucose, sucrose and fructose. OPF juice is expected to address the environmental issues to produce volatile organic compounds (VOCs) especially for production of methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate as OPF is abundantly available as a biomass and easily available throughout Malaysia. The utilization and development of methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate production fungus have been of increasing interest as they are naturally produced without chemical synthesis. *Ceratocystis fimbriata* is a fungus which has the potential for synthesizing esters, it grows quickly and produces a variety of aromas (peach, pineapple, banana, citrus and rose) depending on the strain and culture conditions. The aim of this study was to screen and optimize methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate using a series of experimental design by a head space-solid phase micro extraction (HS-SPME) technique combined with gas chromatography-mass spectroscopy (GC–MS) was used to separate the relative peak area of the compound during the fermentation. Optimization of methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate production are affected by several factors during the period of fermentation. Series of experimental designs were applied to screen and optimize the production of the compound. In the screening study, 2^4 full factorial design were used to find significant factors affecting production of methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate, which are incubation temperature (25 °C-35 °C), initial pH medium (pH4, pH8), agitation speed (100 rpm, 150 rpm) and concentration of glucose (20 g/L, 30 g/L) in OPF juice. The responses in screening were fitted with a multiple linear regression equation and obtained a correlation ($R^2 = 0.8960$) between the experimental data and model data. Then central composite design (CCD) was applied as the experimental design and a polynomial regression model with quadratic term was used to analyze the experimental data using analysis of variance (ANOVA). ANOVA analysis showed that the model was very significant ($p < 0.0001$) for the methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate production. The responses were fitted with the second order polynomial equation with high correlation ($R^2 = 0.9598$) between the observed and predicted values. The results of optimization process showed that a maximum methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate production was obtained under the condition of initial pH medium (8), agitation speed (100 rpm) and incubation temperature (25°C). Under these optimized conditions, the highest 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate production was obtained when the column retention time was 32.80 minutes and the relative peak area was 0.29 % of chromatogram area by using GC-SPME. As a conclusion, this study provides a significant guideline and basic of understanding for the production of methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate compound at larger scale using OPF juice as sole, renewable and sustainable substrate by *C. fimbriata* in the near future.

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LIST OF SYMBOLS

mL	milliliter
L	Liter
kg	kilogram
g	gram
°C	temperature
rpm	Rotation per minute
g/L	Gram per Liter
mL/min	Milliliter per minute
spores/mL	Spores per milliliter

LIST OF ABBREVIATIONS

GC-MS	Gas chromatography mass spectrometry
SPME	Solid phase micro extraction
HPLC	High performance liquid chromatography
ANOVA	Analysis of variance
C. fimbriata	*Ceratocystis fimbriata*
VOCs	Volatile organic compounds
CCD	Central composite design
DOE	Design of experiments
OPF	Oil palm frond
OPT	Oil palm trunk
EFB	Empty fruit bunches
PKC	Palm kernel cake
POME	Palm oil mill effluent
PPF	Palm press fibre
FFB	Fresh fruit bunches
MARDI	Malaysian Agricultural Research and Development Institute
CDW	Cell dry weight
FFD	Full factorial design
RSM	Research surface methodology
PDMS	Polydimethylsiloxane
CAR	Carboxen
DVB	Divinylbenzene
MPOB	Malaysian palm oil board
PKS	Palm kernel shells
MF	Mesocarp fibres
NaOH	Sodium hydroxide
HCL	Hydrochloric acid
MSM	Mineral salt medium

$(NH_4)_2SO_4$	Ammonium sulfate
KH_2PO_4	Monopotassium phosphate
$Ca(NO_3)_2.4H_2O$	Calcium nitrate tetrahydrate
$MgSO_4.7H_2O$	Magnesium sulfate heptahydrate
$Fe(NO_3)_3.9H_2O$	Iron(III) nitrate nonahydrate
$ZnSO_4.7H_2O$	Zinc sulfate heptahydrate
$(MnSO_4.4H_2O)$	Manganese(II) sulfate tetrahydrate
MSM	Mineral salt medium
ATCC	American type of culture collection
PDA	Potato dextrose agar

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